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     FILE 'MEDLINE' ENTERED AT 10:08:59 ON 31 JAN 2001
L1           705 S ADENIVIRUS OR AAV
L2            46 S CAP AND REP
L3         11095 S HOST CELL?
L4         28285 S INDUCIBLE OR CONSTITUTIVE PROMOTER?
L5             3 S L4 AND L2
L6             3 S L5 AND L1
L7             0 S L6 AND L3
L8            13 S TRANSGENE? AND ITR
L9            15 S TRANSGENE? AND ITR?
L10           10 S L9 AND L1
L11            1 S L10 AND L2
L12       134839 S VECTOR? OR PLASMID?
L13          425 S E1A AND E1B
L14          106 S L12 AND L13
L15            7 S L14 AND L4
                 E GAO G/AU
L16            2 S E3 AND L1
L17            0 S WILSON JAMES/AU
L18          493 S WILSON J M/AU
                 E WILSON J M/AU
L19           14 S E3 AND L1
L20            2 S L19 AND L2
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DOCUMENT NUMBER: 96190587
TITLE: Recruitment of wild-type and recombinant adeno-associated virus into adenovirus replication centers.
AUTHOR: Weitzman M D; Fisher K J; **Wilson J M**
CORPORATE SOURCE: Institute for Human Gene Therapy, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, USA.
SOURCE: JOURNAL OF VIROLOGY, (1996 Mar) 70 (3) 1845-54. Journal code: KCV. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199608

AB Replication of a human parvovirus, adeno-associated virus (**AAV**), is facilitated by coinfection with adeno-virus to provide essential helper functions. We have used the techniques of in situ hybridization and immunocytochemistry to characterize the localization of **AAV** replication within infected cells, Previous studies have shown that adenovirus establishes foci called replication centers within the nucleus, where adenoviral replication and transcription occur. Our studies indicate that **AAV** is colocalized with the adenovirus replication centers, where it may utilize adenovirus and cellular proteins for its own replication. Expression of the **AAV** Rep protein inhibits the normal maturation of the adenovirus centers. Similar experiments were performed with recombinant **AAV** (rAAV) to establish a relationship between intranuclear localization and rAAV transduction. rAAV efficiently entered the cell, and its genome was faintly detectable in a perinuclear distribution and was mobilized to replication centers when the cell was infected with adenovirus. The recruitment of the replication-defective genome into the intranuclear adenovirus domains resulted in enhanced transduction. These studies illustrate the importance of intracellular compartmentalization for such complex interactions as the relationship between **AAV** and adenovirus.

ACCESSION NUMBER: 97088284 MEDLINE
DOCUMENT NUMBER: 97088284
TITLE: A novel adenovirus-adeno-associated virus hybrid vector that displays efficient rescue and delivery of the **AAV** genome.
AUTHOR: Fisher K J; Kelley W M; Burda J F; **Wilson J M**
CORPORATE SOURCE: Institute for Human Gene Therapy, University of Pennsylvania Medical Center, Philadelphia, USA.
SOURCE: HUMAN GENE THERAPY, (1996 Nov 10) 7 (17) 2079-87. Journal code: A12. ISSN: 1043-0342.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY WEEK: 19970502

AB Adenovirus and adeno-associated virus (**AAV**) are eukaryotic DNA viruses being developed as vectors for human gene therapy. The strengths of each system have been exploited in a novel vector that is based on an adenovirus-**AAV** hybrid virus incorporated into a plasmid-based molecular conjugate. Efficient rescue and replication of the recombinant **AAV** genome in this hybrid required transient expression of rep. This feature was incorporated into the transducing particle by conjugating a rep expression plasmid to the hybrid virus through a polylysine bridge. The resulting particle is an attractive vehicle for gene therapy because it is easily manufactured and capable of efficiently transducing cells with the end result being rescue and replication of the recombinant **AAV** genome. This particle is also useful in the production of recombinant **AAV** resulting in yields 10-fold greater than that achieved with transfection-based protocols.

DOCUMENT NUMBER: 99044999
TITLE: High-titer adeno-associated viral vectors from a **Rep/Cap** cell line and hybrid shuttle virus.
AUTHOR: Gao G P; Qu G; Faust L Z; Engdahl R K; Xiao W; Hughes J V; Zoltick P W; **Wilson J M**
CORPORATE SOURCE: Institute for Human Gene Therapy, Department of Molecular and Cellular Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA.
CONTRACT NUMBER: P01 AR/NS43648-03 (NIAMS)
P01 HD32649-04 (NICHD)
P30DK47757-05 (NIDDK)
+
SOURCE: HUMAN GENE THERAPY, (1998 Nov 1) 9 (16) 2353-62.
Journal code: A12. ISSN: 1043-0342.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903

AB Adeno-associated virus (**AAV**) is a potential vector for in vivo gene therapy. A critical analysis of its utility has been hampered by methods of production that are inefficient, difficult to scale up, and that often generate substantial quantities of replication-competent **AAV**. We describe a novel method for producing **AAV** that addresses these problems. A cell line, called B50, was created by stably transfecting into HeLa cells a **rep/cap**-containing plasmid utilizing endogenous **AAV** promoters. Production of **AAV** occurs in a two-step process. B50 is infected with an adenovirus defective in E2b, to induce **Rep** and **Cap** expression and provide helper functions, followed by a hybrid virus in which the **AAV** vector is cloned in the E1 region of a replication-defective adenovirus. This results in a 100-fold amplification and rescue of the **AAV** genome, leading to a high yield of recombinant **AAV** that is free of replication-competent **AAV**. Intramuscular injection of vector encoding erythropoietin into skeletal muscle of mice resulted in supraphysiologic levels of hormone in serum that was sustained and caused polycythemia. This method of **AAV** production should be useful in scaling up for studies in large animals, including humans.

DOCUMENT NUMBER: 99214338
TITLE: Gene therapy vectors based on adeno-associated virus type 1.
AUTHOR: Xiao W; Chirmule N; Berta S C; McCullough B; **Gao G** ; Wilson J M
CORPORATE SOURCE: Institute for Human Gene Therapy and Departments of Molecular and Cellular Engineering and of Medicine, University of Pennsylvania, and The Wistar Institute, Philadelphia, Pennsylvania 19104, USA..
cousc@mri.sari.ac.uk
CONTRACT NUMBER: P30 DK47757-06 (NIDDK)
PO1 HD32649-04 (NICHD)
SOURCE: JOURNAL OF VIROLOGY, (1999 May) 73 (5) 3994-4003.
Journal code: KCV. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-AF063497
ENTRY MONTH: 199907
ENTRY WEEK: 19990704
AB The complete sequence of adeno-associated virus type 1 (**AAV**-1) was defined. Its genome of 4,718 nucleotides demonstrates high homology with those of other **AAV** serotypes, including **AAV**-6, which appears to have arisen from homologous recombination between **AAV**-1 and **AAV**-2. Analysis of sera from nonhuman and human primates for neutralizing antibodies (NAB) against **AAV**-1 and **AAV**-2 revealed the following. (i) NAB to **AAV**-1 are more common than NAB to **AAV**-2 in nonhuman primates, while the reverse is true in humans; and (ii) sera from 36% of nonhuman primates neutralized **AAV**-1 but not **AAV**-2, while sera from 8% of humans neutralized **AAV**-2 but not **AAV**-1. An infectious clone of **AAV**-1 was isolated from a replicated monomer form, and vectors were created with **AAV**-2 inverted terminal repeats and **AAV**-1 Rep and Cap functions. Both **AAV**-1- and **AAV**-2-based vectors transduced murine liver and muscle in vivo; **AAV**-1 was more efficient for muscle, while **AAV**-2 transduced liver more efficiently. Strong NAB responses were detected for each vector administered to murine skeletal muscle; these responses prevented readministration of the same serotype but did not substantially cross-neutralize the other serotype. Similar results were observed in the context of liver-directed gene transfer, except for a significant, but incomplete, neutralization of **AAV**-1 from a previous treatment with **AAV**-2. Vectors based on **AAV**-1 may be preferred in some applications of human gene therapy.

DOCUMENT NUMBER: 97083340
TITLE: Novel complementation cell lines derived from human lung carcinoma A549 cells support the growth of E1-deleted adenovirus **vectors**.
AUTHOR: Imler J L; Chartier C; Dreyer D; Dieterle A; Sainte-Marie M; Faure T; Pavirani A; Mehtali M
CORPORATE SOURCE: Transgene, Strasbourg, France.
SOURCE: GENE THERAPY, (1996 Jan) 3 (1) 75-84.
Journal code: CCE. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199706
ENTRY WEEK: 19970601

AB Replication-defective E1-deleted adenoviruses are attractive **vectors** for gene therapy or live vaccines. However, manufacturing methods required for their pharmaceutical development are not optimized. For example, the generation of E1-deleted adenovirus **vectors** relies on the complementation functions present in 293 cells. However, 293 cells are prone to the generation of replication competent particles as a result of recombination events between the viral DNA and the integrated adenovirus sequences present in the cell line. We report here that human lung A549 cells transformed with constitutive or **inducible** E1-expression **vectors** support the replication of E1-deficient adenoviruses. **E1A** transcription was elevated in most of the cell lines, and **E1A** proteins were expressed at levels similar to those of 293 cells. However, the levels of expression of **E1A** did not correlate with the efficiencies of complementation of E1-deleted viruses in A549 clones, since some clones complemented replication in the absence of induction of **E1A** expression. In addition, complementation of E1-deficient adenoviruses did not require expression of the **E1B** 55-kDa protein. Although these cell lines contain the coding and cis-acting regulatory sequences of the structural protein IX gene, they are not able to complement viruses in which this gene has been deleted. In contrast to 293 cells, such new complementation cell lines do not contain the left end of the adenoviral genome and thus represent a significant improvement over the currently used 293 cells, in which a single recombination event is sufficient to yield replication competent adenovirus.

ACCESSION NUMBER: 1998211339 MEDLINE
DOCUMENT NUMBER: 98211339
TITLE: Factors influencing recombinant adeno-associated virus production.
AUTHOR: Salvetti A; Or`eve S; Chadeuf G; Favre D; Cherel Y; Champion-Arnaud P; David-Ameline J; Moullier P
CORPORATE SOURCE: Laboratoire de Therapie Genique, CHU Hotel-DIEU, Nantes, France.
SOURCE: HUMAN GENE THERAPY, (1998 Mar 20) 9 (5) 695-706.
Journal code: A12. ISSN: 1043-0342.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY WEEK: 19980801
AB Recombinant adeno-associated virus (rAAV) is produced by transfecting cells with two constructs: the rAAV vector plasmid and the **rep-cap** plasmid. After subsequent adenoviral infection, needed for rAAV replication and assembly, the virus is purified from total cell lysates through CsCl gradients. Because this is a long and complex procedure, the precise titration of rAAV stocks, as well as the measure of the level of contamination with adenovirus and **rep**-positive **AAV**, are essential to evaluate the transduction efficiency of these vectors in vitro and in vivo. Our vector core is in charge of producing rAAV for outside investigators as part of a national network promoted by the Association Francaise contre les Myopathies/Genethon. We report here the characterization of 18 large-scale rAAV stocks produced during the past year. Three major improvements were introduced and combined in the rAAV production procedure: (i) the titration and characterization of rAAV stocks using a stable **rep-cap** HeLa cell line in a modified Replication Center Assay (RCA); (ii) the use of different **rep-cap** constructs to provide **AAV** regulatory and structural proteins; (iii) the use of an adenoviral plasmid to provide helper functions needed for rAAV replication and assembly. Our results indicate that: (i) rAAV yields ranged between 10(11) to 5 x 10(12) total particles; (ii) the physical particle to infectious particle (measured by RCA) ratios were consistently below 50 when using a **rep-cap** plasmid harboring an **ITR**-deleted **AAV** genome; the physical particle to transducing particle ratios ranged between 400 and 600; (iii) the use of an adenoviral plasmid instead of an infectious virion did not affect the particles or the infectious particles yields nor the above ratio. Most of large-scale rAAV stocks (7/9) produced using this plasmid were free of detectable infectious adenovirus as determined by RCA; (iv) all the rAAV stocks were contaminated with **rep**-positive **AAV** as detected by RCA. In summary, this study describes a general method to titrate rAAV, independently of the **transgene** and its expression, and to measure the level of contamination with adenovirus and **rep**-positive **AAV**. Furthermore, we report a new production procedure using adenoviral plasmids instead of virions and resulting in rAAV stocks with undetectable adenovirus contamination.

DOCUMENT NUMBER: 97139857
TITLE: A stable cell line carrying adenovirus-**inducible** **rep** and **cap** genes allows for infectivity titration of adeno-associated virus vectors.
AUTHOR: Clark K R; Voulgaropoulou F; Johnson P R
CORPORATE SOURCE: Department of Pediatrics, College of Medicine, Children's Hospital Research Foundation, Children's Hospital, Columbus, OH 43205, USA.
SOURCE: GENE THERAPY, (1996 Dec) 3 (12) 1124-32.
Journal code: CCE. ISSN: 0969-7128.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY WEEK: 19970505

AB Adeno-associated virus (**AAV**) vectors are being developed for in vivo and ex vivo gene transfer to human cells. At present, widespread usage of **AAV** vectors is limited primarily by difficulties in generating recombinant virions on a scale sufficient for in-depth preclinical and clinical trials. However, recent work in several laboratories suggests that this technical obstacle should be overcome in the near future. As a result, it can be anticipated that the interest in **AAV** vectors will expand, Thus, it becomes important to develop assay systems that will permit accurate quantification of the infectivity of **AAV** vectors derived from a variety of sources. We have developed an assay using a cell line that expresses **AAV** helper functions (**rep** and **cap**) upon induction by adenovirus infection. This assay system is based on the replication of input rAAV genomes rather than transgene expression (transduction). Thus, infectivity titrations in this system yield an estimation of rAAV infectious particles irrespective of the promoter or transgene present in the vector genome. Moreover, this assay method is more sensitive than conventional methods being used in other l

DOCUMENT NUMBER: 99429612
TITLE: Highly regulated expression of adeno-associated virus large **Rep** proteins in stable 293 cell lines using the Cre/loxP switching system.
AUTHOR: Ogasawara Y; Mizukami H; Urabe M; Kume A; Kanegae Y; Saito I; Monahan J; Ozawa K
CORPORATE SOURCE: Division of Genetic Therapeutics, Jichi Medical School and CREST, Japan Science and Technology Corporation, Tochigi.
SOURCE: JOURNAL OF GENERAL VIROLOGY, (1999 Sep) 80 (Pt 9) 2477-80.
Journal code: I9B. ISSN: 0022-1317.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199912
ENTRY WEEK: 19991202

AB Since the **Rep** proteins of adeno-associated virus (**AAV**) are harmful to cells, it is difficult to obtain stable cell lines that express them constitutively. In this study, stable 293 cell lines were obtained in which large **Rep** expression was **inducible** by using the Cre/loxP switching system. To determine the function of the induced **Rep** proteins, the packaging capacity was examined after supplementation with a plasmid expressing small **Rep** and **Cap** proteins. A significant amount of recombinant **AAV** (5.5 x 10(8) vector particles per 10 cm dish) was produced by transfection with a vector plasmid and infection with Cre-expressing recombinant adenovirus, indicating that the large **Rep** proteins retained the function required for packaging. These findings indicate that large **Rep** protein expression can be strictly regulated by the Cre/loxP system and will also serve as a basis for the development of an efficient **AAV**-packaging cell line.

09/665852: prov: 3/20/98; PCT: 3/18/99

1) A host cell comprising:
A transgene – flanked by ITR's
AAV rep and cap
DNA 1a and E2a genes
all are under the control of regulatory sequences

2) Regulatory sequences comprise different promoters.

3 and 16) first promoter directs expression of E1a gene product.
second promoter directs expression of E1b gene product.
Third promoter directs expression of E2a gene product.

4 and 17) first and third promoters are native, inducible, constitutive (CMV, RSV)

5, 6 and 19 (same)) first and third are the same or different.

18) at least one promoter of the 3 is inducible

7) first and third are inducible

8) first or third are inducible

20) adding two different inducing agents that control expression of gene products

9) transgene, rep/cap, and DNA are integrated into the host chromosome or as an episome, or transiently expressed.

10) transgene and E2a supplied by hybrid adenovirus/AAV

11) transgene supplied by rAAV

12) transgene and E1a/E1b supplied by same vector

13) host cell gets transgene, E1a, E1b, E2a by by hybrid adenovirus/AAV vector. The vector's E1a and E1b are replaced by the transgene and E3 is replaced by E1a and E1b.

14) culturing the host cell of 1, thereby making a rAAV

15) purifying rAAV

21) an rAAV from 14

22) cell lysate substantially free of helper and wt virus

23) rAAV from cell lysate

24) an rAAV with no wt or helper virus.